

Parallel evolution in ichthyophagous annual killifishes of South America and Africa*

by

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ABSTRACT. - Morphological characters of two lineages of aplocheiloid annual killifishes containing predatory species feeding on sympatric congeners were analysed under a phylogenetic framework. They are the South American rivulid genus *Austrolebias* and the African nothobranchiid genus *Nothobranchius*. Independent phylogenetic analyses of morphological characters indicated several apomorphic conditions homoplastically occurring in ichthyophagous species of both genera. These character state transformations may be related to the evolution of ichthyophagous habits. Eleven of these convergent characters are concentrated on the head bones, including jaw suspensorium, jaws and neurocranium. Besides the parallel occurrence of long jaws, convergent transformations involve widening of bone parts, which serve as support to muscles responsible for closing the jaws or retraction and expansion of the mouth cavity. The distribution of the homoplastic conditions among terminal taxa of the two annual fish groups indicates that similar characters states arose at different points of the evolutionary history of South American and African killifishes, with most transformations being gradually acquired along more inclusive clades.

RÉSUMÉ. - Évolution parallèle chez des poissons killis ichtyophages annuels d'Amérique du Sud et d'Afrique.

Dans cette étude, les caractères morphologiques de deux lignées de poissons killis annuels aplocheiloïdes sont analysés dans un cadre phylogénétique contraint. Les deux lignées choisies incluent des espèces ichtyophages cannibales : les rivulides sud-américains du genre *Austrolebias* et les nothobranchiidés du genre africain *Nothobranchius*. Les analyses phylogénétiques séparées des deux lignées, basées sur des caractères morphologiques montrent que plusieurs états apomorphes des espèces ichtyophages sont homoplastiques entre les deux genres. Ces changements parallèles d'états de caractères sont possiblement liés à l'évolution vers un mode alimentaire ichtyophage. Onze de ces caractères sont portés par les os de la tête, en particulier par les os suspenseurs de la mâchoire, par les os de la mâchoire elle-même et par le neurocrâne. En plus de l'allongement des os des mâchoires, on observe des caractères convergents correspondant à l'épaississement des os au niveau des attaches musculaires sollicitées lors de la fermeture de la bouche et lors des mouvements de pro-traction et rétro-traction des mâchoires. La distribution de ces états dérivés homoplastiques chez des taxons terminaux dans deux groupes différents de poissons annuels indique clairement que de mêmes états de caractères sont apparus indépendamment dans l'histoire évolutive des killis en Amérique du Sud d'une part, et en Afrique d'autre part. Enfin, la plupart des modifications associées à l'ichtyophagie sont graduelles dans les groupes où les espèces cannibales sont apparues.

Key words. - Rivulidae - Nothobranchiidae - *Austrolebias* - *Nothobranchius* - Morphology - Osteology - Adaptation - Annualism.

Aplocheiloid killifishes comprise a diversified assemblage of the Cyprinodontiformes, formally divided into three families: the Aplocheilidae, from southern Asia, Seychelles and Madagascar; the Nothobranchiidae, from continental Africa; and, the Rivulidae, from southern North, Central and South America (e.g., Costa, 2004, 2008). Aplocheiloids are small fishes, usually reaching about 40-70 mm of total length, primarily inhabiting freshwater swamps and shallow parts of streams (Amiet, 1987; Costa, 1995, 2006a, 2009a). Independent lineages of nothobranchiids and rivulids (Parenti, 1981; Costa, 1998; Hrbek and Larson, 1999; Murphy *et al.*, 1999) developed a peculiar life style, known as annualism, in which the entire life cycle occurs in seasonal pools and swamps formed only during the rainy season (Myers, 1952; Costa, 2009a). In the dry season all individuals die, while resistant eggs buried in the substrate undergo diapause

until just after the beginning of the next rainy season, when eclosion is followed by rapid development (e.g., Wourms, 1972).

Among the aplocheiloids, the greatest morphological diversification (Costa, 1998, 2011), the most elaborated reproductive behaviour patterns (Costa, 1998; Belote and Costa, 2002, 2003, 2004; Costa *et al.*, 2010) and the most diverse feeding specializations (Costa, 2009b) are concentrated in the annual fish groups. The best examples of such radiations are the South American rivulid genus *Austrolebias* and the African nothobranchiid genus *Nothobranchius*. Although annualism was independently acquired in rivulids and nothobranchiids (Parenti, 1981; Costa, 1998, 2009c), species of both genera inhabit seasonal pools, which are placed in savannah-like environments. *Austrolebias* comprises over 35 species inhabiting temporary pools of central

* Supporting files are available online: <http://www.mnhn.fr/sfi/cybium/numeros/351/sommaire351/05-Costa 699 SF>

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and south-eastern South America (Costa, 2006b), and *Nothobranchius* contains about 50 valid species occurring in temporary pools of central and eastern Africa (e.g., Wildekamp, 2004; Costa, 2008). The sympatric occurrence of three to six congeneric species with distinct morphological patterns and feeding preferences is common in both genera.

Contrasting to the typical diet of annual aplocheiloid fishes usually comprising small invertebrates (Costa, 1995, 2009b; Shibatta and Rocha, 2001), a few species of *Austrolebias* and *Nothobranchius* found in lowlands of south-eastern South America and eastern Africa, areas with great species diversification of those annual fish groups (e.g., Wildekamp, 2004; Costa, 2006b), are ichthyophagous fishes preying on sympatric congeners of smaller size (Wildekamp and Haas, 1992; Costa, 2009b). Piscivory is known to occur in a single species of *Nothobranchius*, *N. ocellatus* (Seegers) (Wildekamp and Haas, 1992). Entire fish specimens were recorded in the gut content of *Austrolebias elongatus* (Steindachner) and *A. prognathus* (Amato) (Costa, 2009b), whereas a third species, *A. cheradophilus* (Vaz-Ferreira, Sierra-de-Soriano & Scaglia-de-Paulete) may eventually feeds on

fish (Costa, 2009b; Laufer *et al.*, 2009). *Austrolebias elongatus*, *A. prognathus* and *Nothobranchius ocellatus* overpass 130 mm of total length, constituting the largest species of the Aplocheiloidei. Besides living in similar habitats, being ichthyophagous and reaching an unusual large size, these species also share a general appearance (i.e., pike-like form). All this suggests that they had parallel evolution under similar adaptive pressures.

Some questions may help to evaluate this parallel evolution towards piscivory among aplocheiloids: 1) which morphological characters have convergently evolved in lineages of ichthyophagous annual fishes? 2) Is the similarity among South American and African ichthyophagous species superficial (i.e., pike-like species reaching large size) or do they share structural similarity leading to greater ability to capture prey? 3) Have morphological character states related to piscivory arisen abruptly at the base of ichthyophagous lineages or did they arise gradually along the base of more inclusive lineages? The use of phylogenies to examine hypotheses of adaptation has been the focus of a series of methodological studies tentatively making a bridge between evolutionary

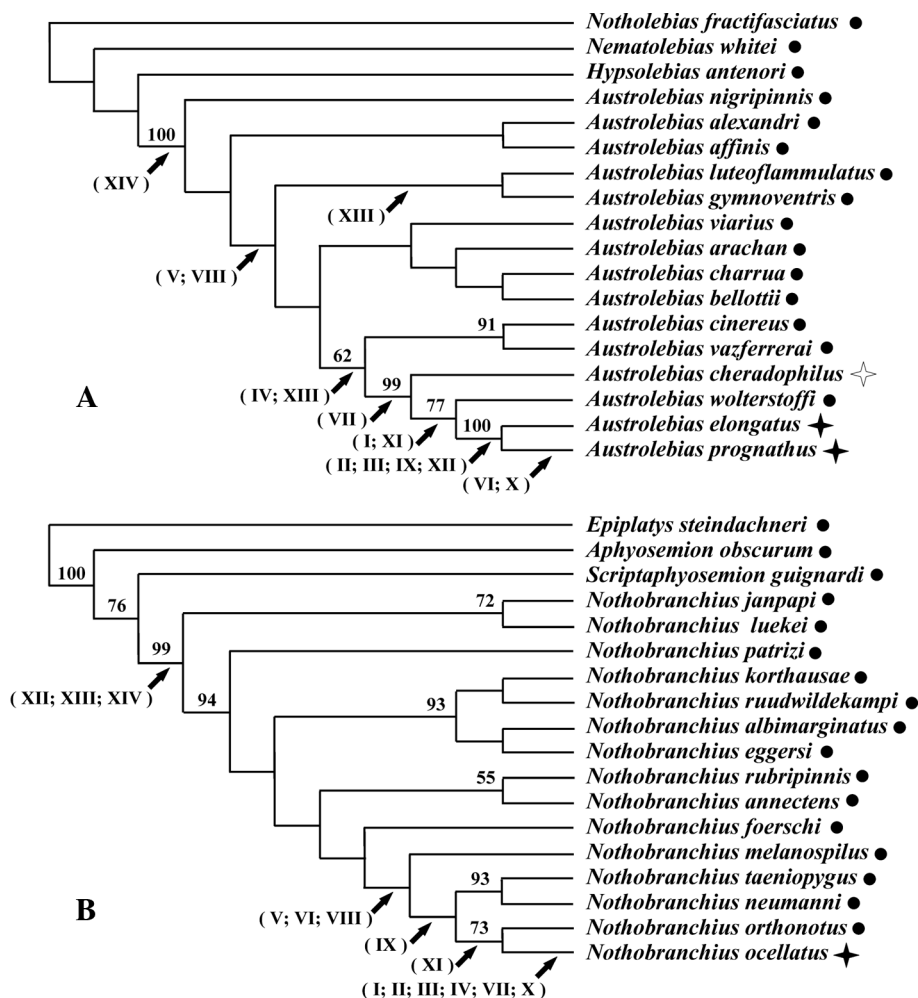


Figure 1. - Most parsimonious phylogenetic trees for morphological characters. **A**: 18 rivulid taxa; **B**: 18 nothobranchiid taxa. Numbers above nodes are bootstrap percentages > 50%; numbers below are derived character states according to table I. Dot after species name indicates non-ichthyophagous species, black star specialized ichthyophagous, and white star sporadic ichthyophagous.

Table I. - Derived character states shared by *Austrolebias* and *Nothobranchius*. Character state numbers are according to appendices 2 and 3 (see supporting files).

Structure	Character state description		Character state number
Jaws	I	Jaws longer than jaw suspensorium	A52.0; N47.0
	II	Medial portion of premaxilla with concavity	A53.1; N48.1
	III	Process of premaxillary alveolar arm rudimentary	A54.1; N49.1
Jaw suspensorium	IV	Anterior portion of entopterygoid not overlapping autopalatine	A47.1; N43.1
	V	Metapterygoid about triangular, upper portion narrow and ventral portion broad	A48.1; N44.1
	VI	Anterodorsal condyle of hyomandibula distinctively longer than posterodorsal condyle	A49.1; N46.1
Neurocranium	VII	Anterior margin of vomer nearly straight to slightly concave	A75.1; N60.1
	VIII	Posterior process of parasphenoid abruptly widening posteriorly	A76.1; N61.1
	IX	Posterior process of parasphenoid reaching posterior portion of basioccipital	A77.1; N62.1
	X	Middle portion of parasphenoid with sharp anterolateral process	A78.1; N63.1
	XI	Lateral process of sphenotic about equal or wider than sphenotic not including lateral process	A79.1; N64.1
Fins	XII	Presence of contact organs on dorsal fin in males	A37.1; N27.1
	XIII	Presence of contact organs on anal fin in males	A38.1; N28.1
	XIV	Medial radials of anal fin cartilaginous or poorly ossified	A85.1; N71.1

pattern and process (e.g., Coddington 1988, 1990; Brooks and McLennan, 1991; Deleporte, 1993; Grandcolas and D'Haese, 2003). Following this framework, two independent phylogenetic analyses based on morphological characters are herein conducted to establish the origin and evolution of convergent morphological structures in ichthyophagous species, the rivulids *Austrolebias elongatus* and *A. prognathus*, and the nothobranchiid *Nothobranchius ocellatus*. The first analysis was directed to the South American rivulid genus *Austrolebias* and the second to the African nothobranchiid genus *Nothobranchius*.

MATERIAL AND METHODS

Phylogenies

The analyses were based on morphological characters taken from Costa (2010) for rivulids and Costa (2009d) for nothobranchiids, where material is listed. Additional material of *Nothobranchius* is listed in appendix 1 (see supporting material). Each analysis encompassed 15 congeners, including both the focal species and species selected to represent all generic lineages, besides three outgroup taxa. Selection of outgroup taxa followed Costa (1998) and Murphy *et al.* (1999), where *Notholebias* (Cynopoecilini) is a basal taxon among cynolebiatines, and Costa (2009c) and Sonnenberg and Busch (2009), where *Epiplatys* is a basal taxon among nothobranchiids. Characters included osteology, latero-sensory system, external morphology and colour patterns.

Character state formulation for both analyses was standardized following Sereno (2007). Character states of all discrete characters were treated as unordered; discrete characters for the analysis of rivulids are listed in appendix 2 and for the analysis of nothobranchiids in appendix 3. The search for most parsimonious trees (traditional search option), bootstrap analysis (1000 replicates), and character state optimizations were performed with TNT 1.1 (Goloboff *et al.*, 2008). Morphometric and meristic data were treated as ordered continuous characters analyzed in TNT analyses according to Goloboff *et al.* (2006). Distribution of characters states of discrete characters among rivulid and nothobranchiid taxa appears in appendices 4 and 5, respectively, and continuous characters in appendices 6 and 7.

Parallel evolution study

Evaluation of parallel evolution was made comparing the distribution of character states independently occurring in both fish families, through: 1) the search of similar character state transformations (cst) occurring in both analyses (including both transformational and neomorphic characters as defined by Sereno, 2007); and 2) mapping the origin of similar cst occurring in each analysis. An index of parallel evolution (PEI) was established to compare the number of homoplastic character states shared by each species of a genus with each species of the opposed genus, measured by $PEI = scst/tcst$, where *scst* is the number of cst shared by both analysed taxa, and *tcst* is the total number of cst shared by both fish groups.

RESULTS

Phylogenies

The most parsimonious phylogenetic trees for the analyses of morphological characters in rivulids and nothobranchiids are illustrated figure 1A and figure 1B, respectively. Both analyses indicate the ichthyophagous species in an apical position within the cladograms. The two ichthyophagous species of *Austrolebias* are grouped into a clade comprising large species with predator appearance, in which these two species form a monophyletic group.

Parallel evolution study

A total of 14 character state transformations occurred independently in *Austrolebias* and *Nothobranchius* (Tab. I; see also figure 1 for origin of each character state). Values of parallel evolution index are given in table II.

Austrolebias prognathus and *N. ocellatus* share all the convergent apomorphic states, thus reaching the highest value of the parallel evolution index (i.e., PEI = 1.00; Tab. II), while a value slightly lower was found when comparing *N. ocellatus* to *A. elongatus* (PEI = 0.87). The value of PEI was relatively low when comparing *A. cheradophilus*, which is a facultative piscivorous species (Laufer *et al.*, 2009) with *N. ocellatus* (PEI = 0.47).

Character state transformations shared by rivulids and nothobranchiids were mainly concentrated in the head (including jaw suspensorium, jaws and neurocranium), encompassing 11 character state transformations (Tab. I). Apomorphic conditions of head bones that independently arose in *Austrolebias* and *Nothobranchius* comprise: jaws long, longer than jaw suspensorium length (Fig. 2B, 2D) (*vs* shorter, Fig. 2A, 2C); a pronounced concavity on the medial portion of the premaxilla (Fig. 2B, 2D) (*vs* concavity absent,

Fig. 2A, 2C); anterior process of the alveolar arm of the premaxilla rudimentary (Fig. 2B, 2D) (*vs* well-developed, Fig. 2A, 2C); reduction of the anterior portion of the entopterygoid, which consequently does not overlap the autopalatine (Fig. 2B, 2D) (*vs* anterior portion of the entopterygoid overlapping autopalatine, Fig. 2A, 2C); ventral portion of metapterygoid widened, making the bone about triangular (Fig. 2B, 2D) (*vs* about rectangular, Fig. 2A, 2C); antero-dorsal condyle of the hyomandibula distinctively longer than posterodorsal condyle (Fig. 2B, 2D) (*vs* condyles about equal in length, Fig. 2A, 2C); anterior margin of the vomer nearly straight to slightly concave (Fig. 3B, 3D) (*vs* convex, Fig. 3A, 3C); posterior process of the parasphenoid abruptly widening posteriorly (Fig. 3B, 3D) (*vs* gradually, Fig. 3A, 3C); posterior process of the parasphenoid reaching the posterior portion of the basioccipital (Fig. 3B, 3D) (*vs* reaching middle portion, Fig. 3A, 3C); middle portion of the parasphenoid with sharp anterolateral process (Fig. 3B, 3D) (*vs* process absent, Fig. 3A, 3C); lateral process of the sphenotic widened, about equal or wider than the sphenotic width (Fig. 3B, 3D) (*vs* conspicuously narrower, Fig. 3A, 3C) (Tab. I). The remaining three shared character state transformations involved morphology of unpaired fins.

DISCUSSION

The phylogenetic analysis support an *Austrolebias* clade containing the four largest species and concentrating apomorphic character states supposedly related to ichthyophagy. This could make unclear if the group formed would be an artefact caused by the high occurrence of convergent features. However, this possibility is discarded by molecular evidence provided by García *et al.* (2002), in a paper

Table II. - Index of parallel evolution among species of *Austrolebias* (first column) and *Nothobranchius* (first line).

	janp	luek	patr	kort	ruud	albi	egge	rubr	anne	foer	mela	taen	neum	orth	ocel
nigr	-	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
alex	-	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
affi	-	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
lute	0.07	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.20	0.20	0.20	0.20	0.20
gymn	0.07	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.27	0.27	0.27	0.27	0.27
viar	-	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.20	0.20	0.20	0.20	0.20
arac	-	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.20	0.20	0.20	0.20	0.20
bell	-	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.20	0.20	0.20	0.20	0.20
char	-	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.20	0.20	0.20	0.20	0.20
cine	0.07	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.27	0.27	0.27	0.27	0.40
vazf	0.07	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.27	0.27	0.27	0.27	0.40
cher	0.07	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.27	0.27	0.27	0.27	0.47
wolt	-	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.20	0.20	0.20	0.20	0.53
elon	0.13	0.20	0.20	0.20	0.20	0.20	0.20	0.13	0.13	0.13	0.27	0.40	0.40	0.47	0.87
prog	0.13	0.20	0.20	0.20	0.20	0.20	0.20	0.13	0.13	0.13	0.33	0.47	0.47	0.53	1.00

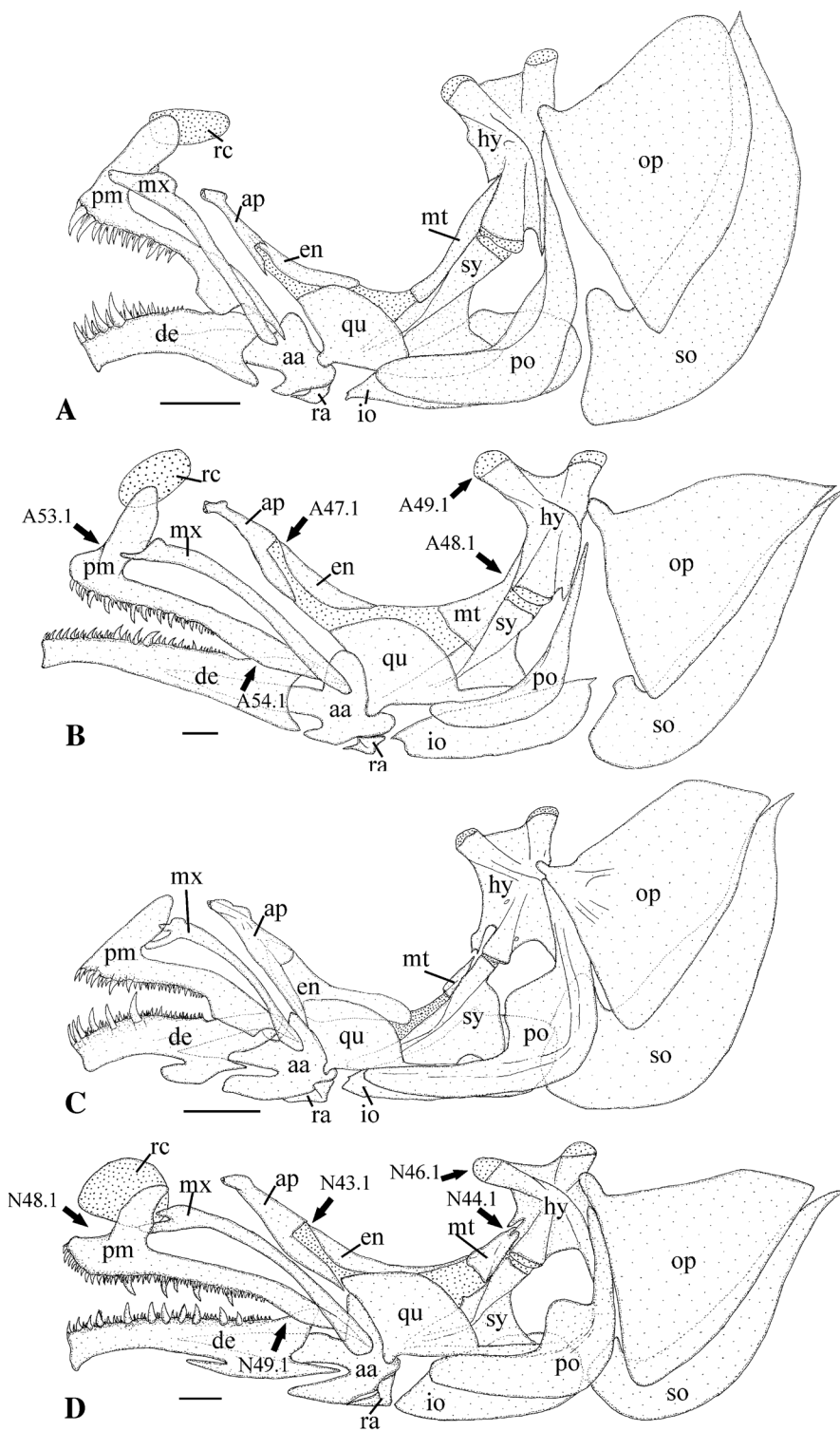


Figure 2. - Jaws, jaw suspensorium and opercular apparatus, left side and lateral view. **A:** *Austrolebias alexandri*; **B:** *Austrolebias prognathus*; **C:** *Nothobranchius albimarginatus*; **D:** *Nothobranchius ocellatus*. aa, angulo-articular; ap, autopalatine; de, dentary; en, entopterygoid; hy, hyomandibula; io, interopercle; mt, metapterygoid; mx, maxilla; op, opercle; pm, premaxilla; po, preopercle; qu, quadrate; ra, retro-articular; rc, rostral cartilage; so, subopercle; sy, symplectic. Meckel's cartilage is not represented; rostral cartilage is not represented in C. Larger stippling indicates cartilage. Scale bar = 1 mm. Arrows indicate apomorphic features related to ichthyophagy; character code follows table I and appendices 2 and 3 (see supporting files).

mostly directed to relationships among Uruguayan species of *Austrolebias*, where that clade comprising large species is strongly supported. The occurrence of all the derived states of characters shared by *Austrolebias* and *Nothobranchius* in the clade comprising *A. elongatus* and *A. prognathus*, and in

N. ocellatus suggests that apomorphic conditions are related to the evolution of ichthyophagous habits.

The similarity among ichthyophagous species of the two aplocheiloid genera analysed is not restricted to their general appearance, since they share a series of similar osteological

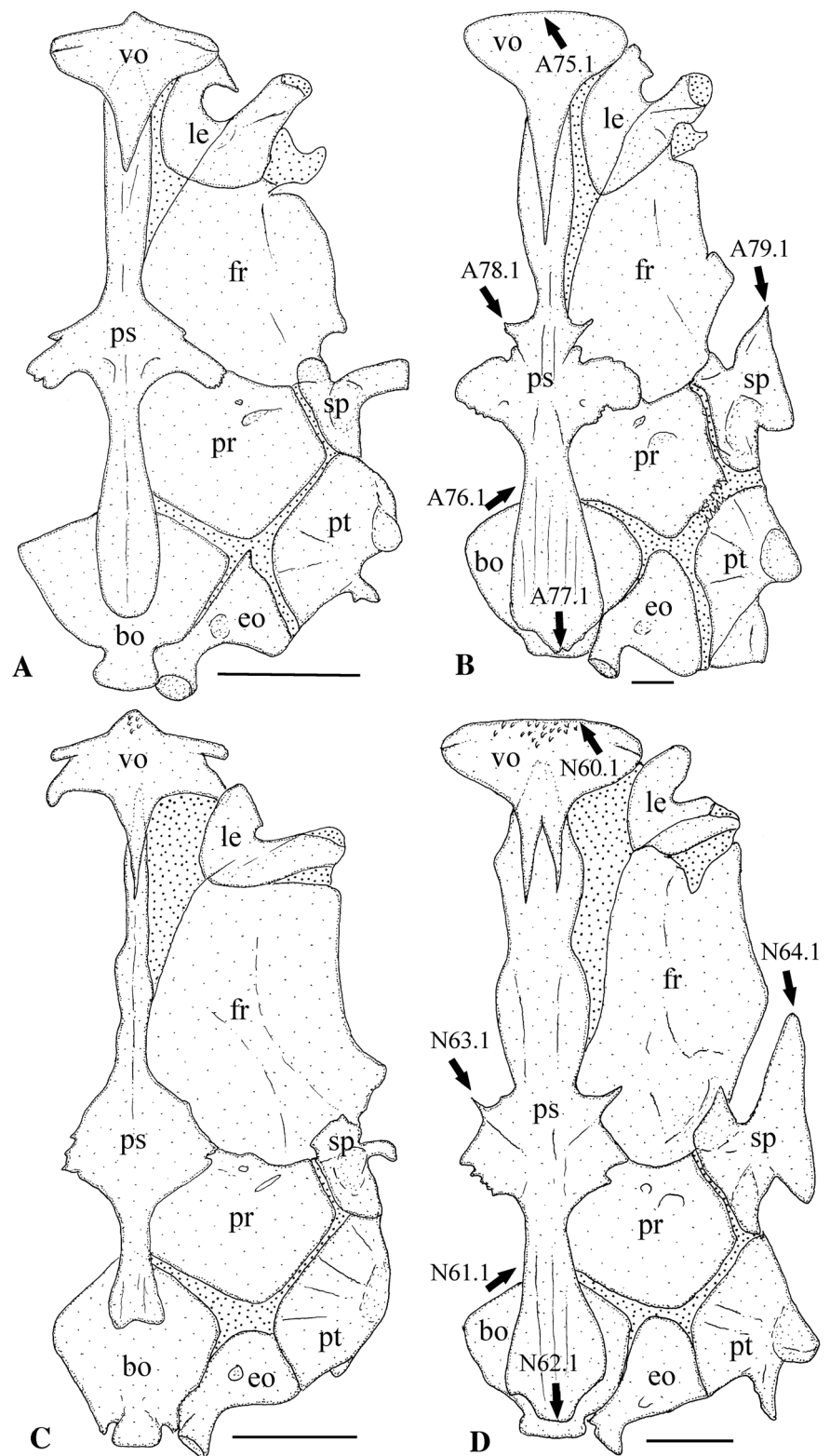


Figure 3. - Neurocranium, ventral view. **A:** *Austrolebias alexandri*; **B:** *Austrolebias prognathus*; **C:** *Nothobranchius luekei*; **D:** *Nothobranchius ocellatus*. bo, basioccipital; eo, exoccipital; fr, frontal; le, lateral ethmoid; pr, prootic; ps, parasphenoid; pt, pterotic; sp, sphenotic; vo, vomer. Larger stippling indicates cartilage. Scale bar 1 mm. Arrows indicate apomorphic features related to ichthyophagy; character code follows table I and appendices 2 and 3 (see supporting files).

features (Figs 2, 3). Most of these parallel acquisitions are concentrated on head bones, whereas a few of them involve morphology of unpaired fins, conditions usually related to

the reproductive behaviour (e.g., Wiley and Collette, 1970). Although mechanisms of prey capture in aplocheiloids, and thus how the head bones function, are still unknown, apo-

morphic conditions shared by ichthyophagous species are predictably related to the evolutionary acquisition of the ichthyophagous habit. The most obvious feature is the lengthening of the jaw bones and elongation of the anterior portion of the jaw suspensorium, which allow the fish to swallow large preys. Other shared osteological characters, such as a concavity on the medial portion of the premaxilla, an abrupt posterior widening on the posterior process of the parasphenoid, a sharp anterolateral process on the middle portion of the parasphenoid, a wide lateral process of the sphenotic, are related to bone areas serving as support for muscles acting during jaw movement and retraction or expansion of the mouth cavity through palatal arc movements. So, some apomorphic modifications in the jaw suspensorium bones and parts of the jaw bones may be associated with the hypertrophied adductor mandibulae muscles in species of the *A. elongatus* group and *N. ocellatus*, which are responsible for closing the jaws and have their origin on the jaw bones and insertion on the posterior part of the jaw suspensorium (e.g., Winterbottom, 1974). Also related to mechanisms involving the palatal arch, the muscle levator arcus palatini has its origin on the lateral process of the sphenotic and insertion on the metapterygoid, and the muscle adductor arcus palatini originates at the parasphenoid and is inserted on the inner portion of the palatal arch, on the metapterygoid.

The distribution of the homoplastic conditions among terminal taxa of the two annual fish groups studied (Fig. 1) indicates some similarities. A wide lower portion of the metapterygoid, a wide posterior portion of the parasphenoid, and a wide lateral process of the sphenotic are found in clades encompassing both specialized ichthyophagous and non-ichthyophagous species, thus considered as originating before ichthyophagy. In both groups, the origin of a wide lateral process of the sphenotic, the support for the levator arcus palatini, precedes the origin of a pronounced concavity on the medial portion of the premaxilla, a rudimentary process of the premaxillary alveolar arm, and a sharp anterolateral process on the middle portion of the parasphenoid. The three latter apomorphies are unique for ichthyophagous taxa, suggesting their origin is contemporaneous or younger than the acquisition of the ichthyophagous habits. On the other hand, the distribution of other apomorphies indicates that similar characters states may have arisen at different points of the evolutionary history of South American and African killifishes, thus presenting incongruent distribution in each annual fish group. A long anterior condyle of the hyomandibula, a long posterior process of the parasphenoid, and the presence of contact organs on male dorsal fin, were restricted to the ichthyophagous taxa of South American annual killifishes, but were widespread among several taxa, both ichthyophagous and non-ichthyophagous, in the African killifishes; in contrast, a shortened entopterygoid and a concave or straight anterior margin of the vomer were uniquely found in the sin-

gle African ichthyophagous annual killifish, whereas those same character states occurred in both ichthyophagous and non-ichthyophagous among the South American killifishes. These data indicate that among the homoplastic character states shared by South American and African ichthyophagous taxa, some have arisen independently from the establishment of the ichthyophagous diet, with the gradual acquisition of convergent features following different evolutionary path routes.

Acknowledgements. - Special thanks are due to T. Litz, P. Laurino, F.I. Prieto, H. Salvia, J. Salvia, E. Perujo, I. Perujo, and R. Recuero, for collecting and providing excellent collections from Uruguay. I am also grateful to A. Miquelarena and H. López for supporting field studies in Argentina and making available fine collections from that country; R. Filiberto and L. Protogino, for help during collecting trips in Argentina; to C. Bove, B. Costa, P. Bragança and M. A. Barbosa for help in collecting annual fishes in Tanzania; and, to P. Migüel for the loan and exchange of material from Africa. I would like to express my gratitude to C.T. Philbrick for comments and suggestions to the manuscript, and to O. Otero, for editorial support. The manuscript benefited from the suggestions and corrections provided by two reviewers. This study was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - Ministério de Ciência e Tecnologia).

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Reçu le 27 août 2010.

Accepté pour publication le 15 février 2011.

List of supporting files

Available on Cybium website: <http://www.mnhn.fr/sfi/cybium/numeros/351/sommaire351/05-Costa 699 SF>

Appendix 1. - List of additional material examined.

Appendix 2. - Character statements for discrete characters of rivulid taxa.

Appendix 3. - Character statements for discrete characters of nothobranchiid taxa.

Appendix 4. - Data matrix of distribution of character states of discrete characters of rivulid taxa.

Appendix 5. - Data matrix of distribution of character states of discrete characters of nothobranchiid taxa.

Appendix 6. - Data matrix of continuous characters of rivulids.

Appendix 7. - Data matrix of continuous characters of nothobranchiids.